J Nanopart Res (2012) 14:860
DOI 10.1007/s11051-012-0860-2

RESEARCH PAPER

Mycogenic synthesis of silver nanoparticles by the Japanese environmental isolate *Aspergillus tamarii*

Ramasamy Rajesh Kumar · Krishnamurthy Poornima Priyadharsani · Kaliannan Thamaraiselvi

Received: 14 September 2010 / Accepted: 4 April 2012

Abstract In this study, an environmental friendly process for the synthesis of silver nanoparticles (AgNPs) using a fungus *Aspergillus tamarii* has been investigated. The process of silver ion reduction by the fungal extracellular filtrate was spontaneous which lead to the development of an easy process for synthesis of silver nanoparticles. The AgNPs formed were characterized using UV–Visible spectrum, FTIR, and SEM. The results revealed that silver ions reduction by the fungal extracellular filtrate started at 420 nm after 0.5 h of incubation time. The FTIR peaks were observed at 1393, 1820, 2727, and 3545 cm^{-1}. The SEM result showed the distribution of spherical AgNPs ranging from 25 to 50 nm.

Keywords Nanoparticles · Extracellular biosynthesis · *Aspergillus tamarii* · Nanobiotechnology · Silver ions

Introduction

In recent years, bionanotechnology research is emerging as cutting edge technology which requires the collaboration between chemists, physicists, biologists, and engineers. Nanoparticles-particles having one or more dimensions of the order of 100 nm or less-have attracted great attention due to their unusual and fascinating properties (Daniel and Astruc 2004; Kato 2011) and are commonly synthesized using two approaches—top down and bottom up (Fendler 1998). In the first approach, the bulk materials are generally broken down to nano sized particles and in latter, atoms or molecules are assembled to molecular structures in nanometer range which is the chemical and biological synthesis of nanoparticles (Narayanan and Sakthivel 2010). In chemical method, the use of toxic chemicals on the surface of nanoparticles and non polar solvents during the synthesis procedure limits their applications in biomedical and clinical fields. Therefore, there is a need for the development of clean, safe, biocompatible, cost effective, non toxic, sustainable, and environmental friendly method for synthesizing the nanoparticles. Compared with the traditional synthetic methods, biological systems provide a novel idea for the production of nanomaterials (Bansal et al. 2011). The use of the microbial cells which have highly structured physical and biosynthetic activities for the synthesis of nanosized materials has recently emerged as a novel approach for the synthesis of metal nanoparticles (Gericke and Pinches 2006). The process devised by nature for the synthesis of inorganic materials in nano- and micro-length scales have contributed to the development of a relatively new and largely unexplored area of research

R. Rajesh Kumar · K. Poornima Priyadharsani · K. Thamaraiselvi (✉)
Laboratory of Molecular Microbial Bioremediation and Nanobiotechnology, Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli 620 024, Tamilnadu, India
e-mail: kthamaraiselvi@hotmail.com

Published online: 01 May 2012

which is based on the application of microbes in the biosynthesis of nanomaterials (Sastry et al. 2004). Microorganisms such as algae, bacteria, and fungi produce nanoparticles either intracellularly or extracellularly (Ingle et al. 2008) and also some plant materials (Mohanpuria et al. 2008) are used for the synthesis of nanoparticles. The application of fungi in the synthesis of nanoparticles is a relatively exciting since they are fastidious to grow, secrete large amounts of enzymes and are simpler to deal in the laboratory. Fungi are more advantageous, since the fungal mycelia mesh can withstand flow pressure, agitation, and other conditions in bioreactors or other chambers compared to other microbes and plant materials. Synthesis of nanoparticles outside the cell (extracellularly) has many applications as it is void of unnecessary adjoining cellular components from the cell. Mostly fungi are regarded as the organisms that produce nanoparticles extracellularly because of their enormous secretary components, which are involved in the reduction and capping of nanoparticles. Fungal species have been reported for the synthesis of nanoparticles such as silver, gold, platinum, silicon, titanium, zirconium, barium, bismuth, cadmium etc., (Mohanpuria et al. 2008; Narayanan and Sakthivel 2010). Among them silver nanoparticles have wide applications and are employed as spectrally selective coating for solar energy absorption, optimal receptors in intercalation material for electrical batteries, polarizing filters, catalysts in chemical reaction, biolabelling, and as antimicrobial agents in biomedical field. Studies on the extracellular synthesis of silver nanoparticles using fungal species such as *A. fumigatus* (Bhainsa and D'Souza 2006), *P. chrysoporium* (Vigneshwaran et al. 2006), *Fusarium solani* (Ingle et al. 2009), *F. acuminatum* (Ingle et al. 2008), *T. asperellum* (Mukherjee et al. 2008), *P. fellutatum* (Kathiresan et al. 2009), *P. brevicompactum* (Shaligram et al. 2009), *C. cladosporoides* (Balaji et al. 2009) have been reported. The present study focuses on the application of *A. tamarii* for the extracellular synthesis of AgNPs because this fungus has not been explored for the formation and stabilization of AgNPs. Extracellular synthesis of AgNPs has been monitored using UV–Vis spectrophotometer, the protein—AgNPs interaction examined by FTIR, the crystalline nature of AgNPs studied by X-Ray diffraction, size and morphology of the AgNPs analyzed using SEM.

Experimental procedure

The fungi *Aspergillus tamarii* (MAFF 111736) was obtained from Gene bank, National Institute of Agrobiological Sciences (NIAS), Japan and maintained in potato dextrose slant at −80 °C. All analytical reagents and media were obtained from Qualigens (Mumbai, India) and Himedia (Mumbai, India). Fungal biomass used for biosynthetic experiments was grown aerobically in liquid growth medium which contained (g/L): KH_2PO_4, 7.0; K_2HPO_4, 2.0; $MgSO_4{\cdot}7H_2O$, 0.1; $(NH_4)_2SO_4$, 1.0; yeast extract, 0.6 and glucose, 10.0 (Bhainsa and D'Souza 2006). The flasks were inoculated with culture and incubated in an orbital shaker (80 rpm) for 72 h at 25 °C. After three days of incubation, the fungal biomass was harvested using plastic sieve and then washed extensively with distilled water to remove any medium components. Fresh clean biomass was weighed, transferred to 200 mL of MilliQ water and incubated in the shaker (80 rpm) for 72 h at 25 °C. Then, the biomass was filtered through Whatman filter paper No. 1 and the cell free filtrate was used in experiments. To the 50 mL of cell filtrate in 250 mL conical flask, carefully weighed quantity (8.4 mg) of silver nitrate 1 mM was mixed and agitated in shaker (80 rpm) at 25 °C in dark. Control without the $AgNO_3$ (cell free filtrate) was also kept at the same conditions as described above. Samples were withdrawn at predetermined time intervals (0, 0.5, 2, 6, 24, 48, 72 h) and the spectra was recorded from 200 to 800 nm using UV–Vis spectrophotometer (Optizen 2120 UV, Mecasys, Korea). After 72 h of incubation the cell free medium amended with $AgNO_3$ after lyophilization was used for the characterization of synthesized nanoparticles by Fourier transform infrared spectroscopy in the region of 4,000–400 cm^{-1} (Perkin Elmer, USA), X-ray diffraction (Rigaku D/Max ULTIMA11, Japan) analysis in 0–1,400 at 2θ angle and scanning electron microscopy (Carl Zeiss, SIGMA, UK).

Results and discussion

The harvested fungi *A. tamarii* weighed about 8 g (wet weight) after growing in the biomass growth medium. The fungal biomass after incubation for 72 h with Milli Q water was separated by filtration. The filtrate

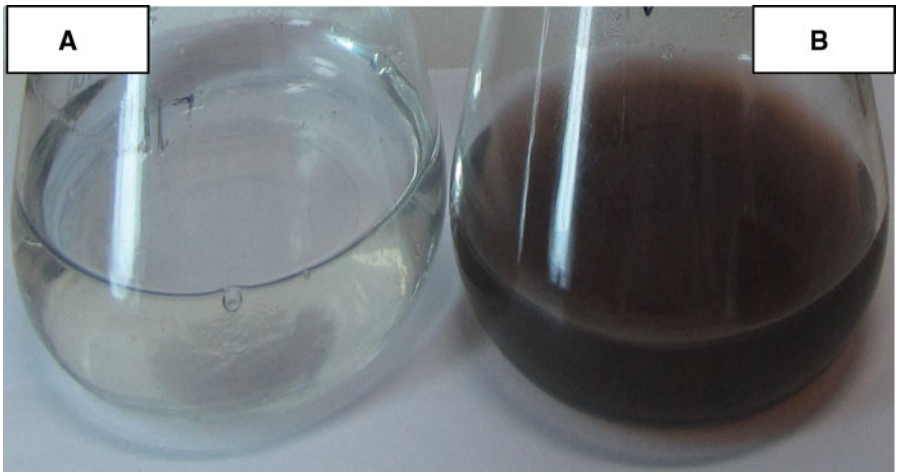


Fig. 1 Conical flasks of extracellular filtrate of *A. tamarii* after 72 h at 25 °C in dark condition **A** Cell filtrate without silver ion (control) **B** Cell filtrate with silver ion

incubated without silver ions (control) did not show any color change in Fig. 1A, whereas the filtrate with 1 mM silver ions incubated in dark environmental conditions showed gradual change from transparent white color to intense brown color in Fig. 1B. The appearance of the brown color was observed after 24 h incubation, and this an indication of formation of colloidal AgNPs in the medium. The brown color of the medium would be due to the excitations of surface resonance and deposition of AgNPs (Mulvaney 1996; Ahmad et al. 2003) or interband transition particularly on the size of effect (Fayaz et al. 2009). The absorption spectrum of the medium containing the silver ions showed maximum intensity at 420 nm after 0.5 h of incubation in Fig. 2. It has been reported that homogenous silver nanoparticles can be produced at the surface plasmon resonance band at ~420 nm (Ahmad et al. 2003) and also it has been reported that the formation of AgNPs depends on the dielectric constant of medium, size of the particle etc. (Balaji et al. 2009). The resonance band at 420 nm is due to the formation of homogenous spherical AgNPs (Vigneshwaran et al. 2007). The concentration of the silver ion solution and the enzymes released by the fungus *A. tamarii* are responsible for the biosynthesis of AgNPs with different crystalline structures. The peak at 420 nm with high absorbance is specific for AgNPs and similar peak was also observed in the AgNPs synthesized by *Aspergillus fumigatus* (Bhainsa and D'Souza 2006), *Penicillium brevibacterium* (Shaligram et al. 2009), *Aspergillus flavus* (Vigneshwaran et al. 2007), *Fusarium oxysporum* (Ahmad et al. 2003), and *F. solani* (Ingle et al. 2008). With the increased incubation time, the absorbance also increased which could be due to the reduction of silver ions present in the aqueous solution which results in the increased number of AgNPs (Bhainsa and D'Souza 2006) and the shape of the particles could be deviation from the ideal spherical geometry (Vigneshwaran et al. 2006). The result indicates that the Ag^{+} had been reduced to Ag^{0} after 0.5 h contact with the fungus filtrate of *A. tamarii*.

FTIR spectroscopy is a useful tool for quantifying secondary structure in the metal nanoparticle—protein interaction by the absorption of infra red (IR) radiation through resonance of non-centro symmetric (IR active) modes of vibration. The FTIR spectrum of a control and sample were compared. The spectrum of the control is shown in Fig. 3, displayed a peak at 3,400 and 3,467 cm^{-1} for primary amines, the stretching vibration between N–H was reported at 1,637 cm^{-1}, whereas a peak at 1,236 cm^{-1} represented C–O stretching of aromatic ethers. The peak at 1,088 cm^{-1} showed aliphatic amines with C–N vibration. The peak at 686 cm^{-1} showed –C–H stretching vibration. The FTIR spectrum of the synthesized silver nanoparticles after 72 h of incubation showed significant change in the position of the peaks, when compared to the control. The FTIR peaks were obtained at 1,393.35, 1,820.12, 2,727.98, and 3,545.59 cm^{-1} which is shown in Fig. 4. The peaks at 3,545.59 and 2,727.98 cm^{-1} corresponds to the stretching vibrations of primary and secondary

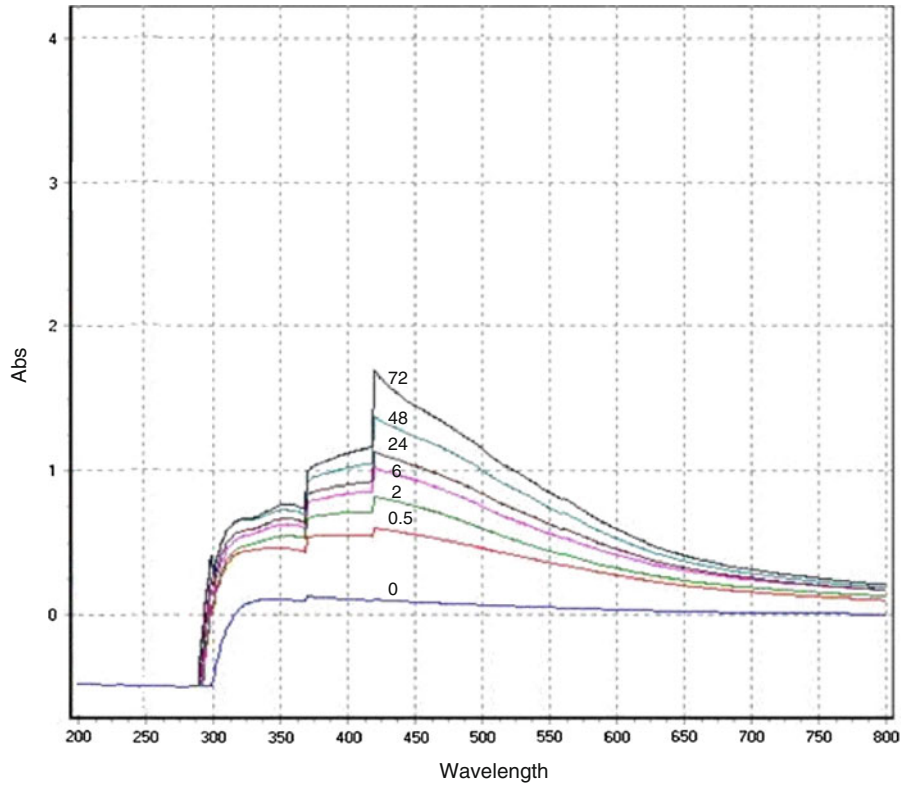
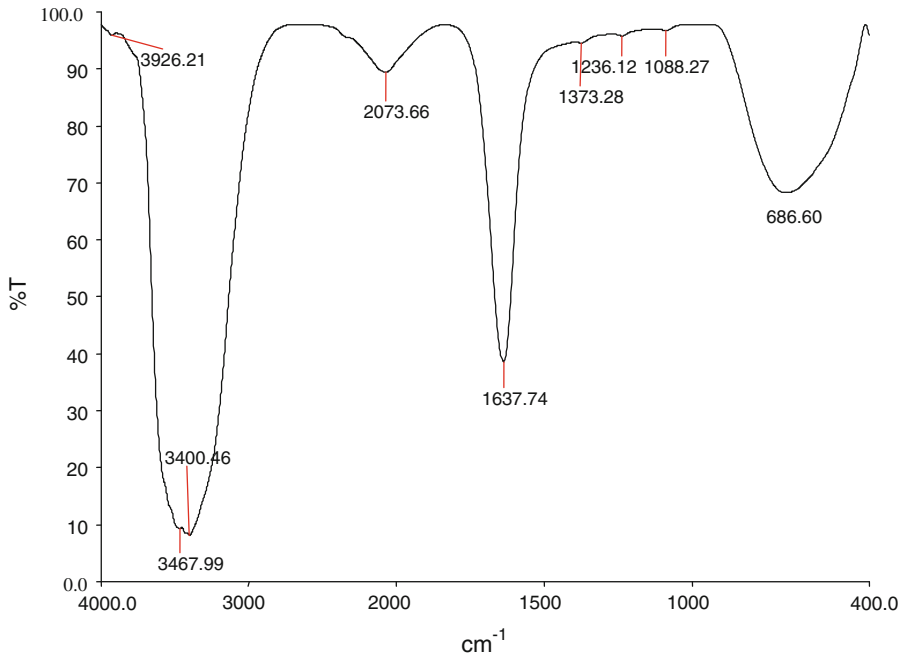


Fig. 2 UV–Vis spectra of fungal extracellular filtrate amended with $AgNO_3$ as a function of time

amines while their bending vibrations are seen at 1,820.12 cm^{-1} and the peak of 1,393.35 cm^{-1} corresponds to an assigned functional group of residual NO_3^- (Luo et al. 2005). The exact mechanism involved in the formation of AgNPs has to be explored. However, there are several reports which elucidate the formation or biosynthesis of AgNPs. Mainly proteins are involved in the biosynthesis of nanoparticles (Kalimuthu et al. 2008). The free amine groups of cysteine residues in the proteins bind to nanoparticles and thereby stabilizes the AgNPs (Gole et al. 2001; Mandal et al. 2005). The role of NADH-dependent nitrate reductase in the silver ion reduction process has been reported (Ahmad et al. 2003; Kalimuthu et al. 2008), where NADH acts as an electron carrier (Shaligram et al. 2009). The carbonyl group and peptides of proteins have stronger ability to bind silver ions (Balaji et al. 2009). During the process, proteins probably form a coat on the nanoparticles so as to avoid agglomeration of particles, thereby stabilizing the nanoparticles. With all these analysis we can infer that the AgNPs are thus stabilized in a solution by a capping agent, likely to be surface bound proteins secreted by *A. tamarii*.

XRD pattern shows intense diffraction peaks at 33°, 45°, and 62°, 2θ may be indexed to the (111), (200), and (220) planes of the face-centered cubic (fcc) silver, respectively. The face-centered cubic unit cell is a cube (all sides of the same length and all face perpendicular to each other) with an atom at each corner of the unit cell and an atom situated in the middle of each face of the unit cell. Similar results were observed by Vigneshwaran et al. (2007), Philip (2009), and Shaligram et al. (2009). The diffraction peak corresponding to the (111) plane is more intense than the other planes. The intensity ratio between (200) and (111) diffraction is much lower than the visual value infers that the (111) plane is predominant orientation (Kannan and John 2008).

The SEM visualization enables to measure the size and shape of the AgNPs formed. The images of the SEM results are shown in the Fig. 5. The silver



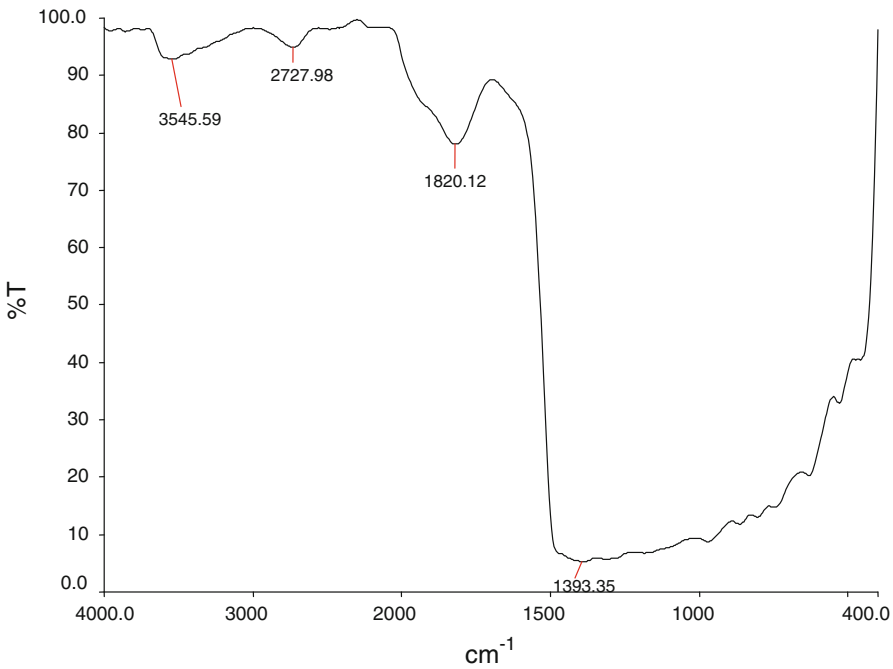


Fig. 3 FTIR spectra for extracellular filtrate of *A. tamarii* (control)

Fig. 4 FTIR spectra of freeze-dried powder of AgNPs formed after 72 h incubation of extracellular filtrate of *A. tamarii* with 1 mM $AgNO_3$ (experiment)

nanoparticles synthesized were spherical in shape and showed a large distribution of sizes in the range of 25–50 nm. AgNPs synthesized by *F. oxysporum* (Duran et al. 2005) and *Penicillium* strain (Sadowski et al. 2008) using the SEM has been reported. The nanoparticles synthesized did not show direct contact within aggregates and the stabilization of nanoparticles occurs through a capping agent.

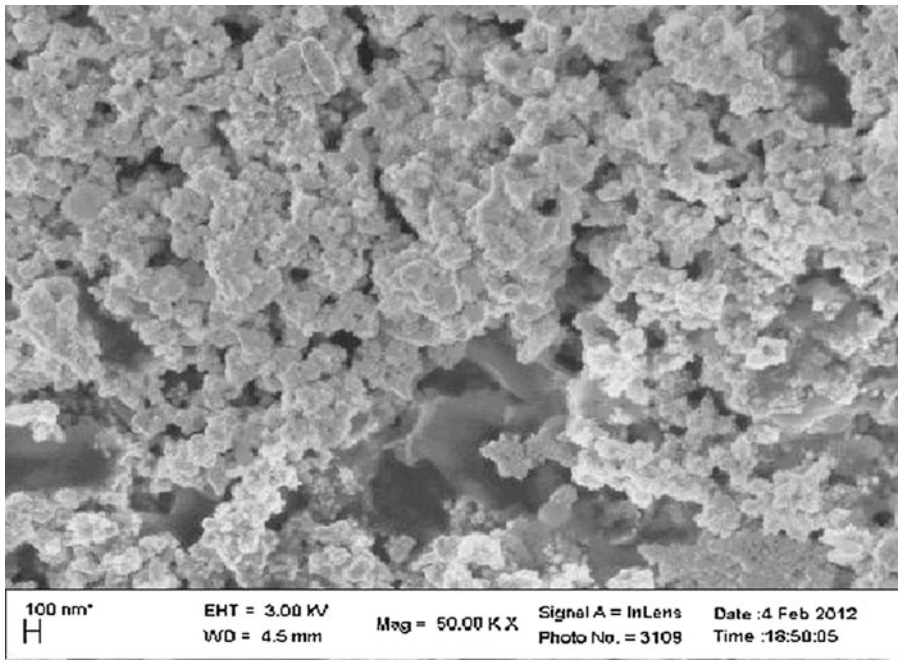


Fig. 5 Scanning electronic micrograph of the freeze-dried powder of AgNPs formed after 72 h incubation of extracellular filtrate of *A. tamarii* with 1 mM $AgNO_3$

Conclusion

In this study we demonstrated and achieved a simple, spontaneous, stable biogenic method of AgNPs using the extracellular filtrate of fungus *A. tamarii* which is alternative to chemical synthesis process. The UV absorption spectra at 420 nm confirmed the reduction of silver ions after 30 min of incubation time. FTIR results infer that the AgNPs stabilized in a solution by a capping agent, likely to be surface bound proteins secreted by the fungus. SEM analysis revealed the size of AgNPs ranging from 25–50 nm. XRD confirmed the crystalline face centered cubic (fcc) AgNPs. Biosynthesized silver nanoparticles will be used for the remediation of pollutants and the work is also under progress. Further, we are trying to evaluate the efficiency of this fungus for the synthesis of AgNPs, AuNPs, and PtNPs in large scale production in a bioreactor.

Acknowledgments The authors acknowledge Gene bank, National Institute of Agrobiological Sciences (NIAS), Japan for providing the fungal culture and also Dr. K. Jeganathan, Coordinator, Centre for Nanoscience and Nanotechnology, Bharathidasan University, Tiruchirappalli, India for providing SEM facility. One of author Rajesh kumar acknowledges UGC for providing Rajiv Gandhi National Research Fellowship.

References

Ahmad A, Mukherjee P, Mandal D, Senapati S, Khan MI, Kumar R, Sastry MJ (2003) Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloid Surf B 28:313–318

Balaji DS, Basavaraja S, Deshpande R, Mahesh BD, Prabhakar BK, Venkataraman A (2009) Extracellular biosynthesis of functionalized silver nanoparticles by strains *Cladosporium cladosporoides* fungus. Colloids Surf B Biointerfaces 68:88–92

Bansal V, Ramanathan R, Bhargava SK (2011) Fungus-mediated biological approaches towards "green" synthesis of oxide nanomaterials. Aust J Chem 64:279–293

Bhainsa KC, D'Souza SF (2006) Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigates*. Colloids Surf B Biointerfaces 47:160

Daniel MC, Astruc D (2004) Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chem Rev 104(1):293–346

Duran N, Marcato PD, Alves OL, De Souza GIH, Esposito E (2005) Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. J Nanobiotechnol 3(8):1–7

Fayaz AM, Balaji K, Kalaichelven PT, Venkatesan R (2009) Fungal based synthesis of silver nanoparticles: an effect of temperature on the size of particles. Colloids Surf B Biointerfaces 74:123–126

Fendler JH (ed) (1998) Nanoparticles and nanostructured films: preparation, characterization and applications. Wiley, Weinheim

Gericke M, Pinches A (2006) Biological synthesis of metal nanoparticles. Hydrometallurgy 83:132–140

Gole A, Dash C, Ramakrishnana V, Sainkar SR, Mandale AB, Rao M, Sastry M (2001) Pepsin-gold colloid conjugates: preparation, characterization, and enzymatic. Langmuir 17:1674–1679

Ingle A, Gade A, Pierrat S, Sonnichsen C, Rai M (2008) Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria. Curr Nanosci 4:141–144

Ingle A, Rai M, Gade A, Bawaskar M (2009) *Fusarium solani*: a novel biological agent for the extracellular synthesis of silver nanoparticles. J Nanopart Res 11:2079–2085

Kalimuthu K, Babu RS, Venkataraman D, Bilal M, Gurunathan S (2008) Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. Colloids Surf B Biointerfaces 65:150–153

Kannan P, John SA (2008) Synthesis of mercapto thiadiazole functionalized gold nanoparticles and their self-assembly on Au substrates. Nanotech 19:0850602

Kathiresan K, Manivanan S, Nabeel MA, Dhivya B (2009) Studies on silver nanoparticles synthesized by a marine fungus, *Penicillium fellatanum* isolated from coastal mangrove sediment. Colloids Surf B Biointerfaces 71:133–137

Kato H (2011) In vitro assays: tracking nanoparticles inside cells. Nat Nanotechnol 6:139–140

Luo L, Yu S, Qian S, Zhou T (2005) Large-scale fabrication of flexible silver/cross linked poly (vinyl alcohol) coaxial nanoscale by a facial solution approach. J Am Chem Soc 127:2822–2823

Mandal S, Phadtre S, Sastry M (2005) Interfacing biology with nanoparticles. Curr Appl Phys 5:118–127

Mohanpuria P, Rana NK, Yadav SK (2008) Biosynthesis of nanoparticles: technological concepts and future applications. J Nanopart Res 10:507–517

Mukherjee P, Roy M, Mandal BP, Dey GK, Mukherjee PK, Ghatak J et al (2008) Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*. Nanotechnology 19:075103

Mulvaney P (1996) Surface plasmon spectroscopy of nanosized metal particles. Langmuir 12:788–800

Narayanan KD, Sakthivel N (2010) Biological synthesis of metal nanoparticles by microbes. Adv Colloid Interf Sci 156:1–13

Philip D (2009) Biosynthesis of Au, Ag and Au–Ag nanoparticles using edible mushroom extract. Spectrochim Acta Part A 73:374–381

Sadowski Z, Maliszewska IH, Grochowalska B, Polowczyk I, Kozlecki T (2008) Synthesis of silver nanoparticles using microorganisms. Mater Sci Poland 26:2419–2424

Sastry M, Ahmad A, Khan MI, Kumar R (2004) Microbial nanoparticle production. In: Niemeyer CM, Mirkin CA (eds) Nanobiotechnology. Wiley, Weinheim, pp 126–135

Shaligram NS, Bule M, Bhambure R, Singhal RS, Singh SK, Szakacs G et al (2009) Biosynthesis of silver nanoparticles using the aqueous extract from the compaction producing fungal strain. Process Biochem 44:939–943

Vigneshwaran N, Kathe AA, Varadarajan PV, Nachane RP, Balasubramanya RH (2006) Biomimetics of silver nanoparticles by white rot fungus, *Phaenerochaete chrysosporium*. Colloids Surf B Biointerfaces 53:55–59

Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanya RH (2007) Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. Mater Lett 61:1413–1418